Cyclic GMP Production by ANP, BNP, and NO during Worsening and Improvement of Chronic Heart Failure

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SUMMARY

Cyclic GMP (cGMP) serves as an intracellular second messenger of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and nitric oxide (NO) and its peripheral blood concentration is an index of its biological activity. It has been reported that the plasma concentration of cGMP is correlated with the concentrations of ANP and BNP and is related to the prognosis of chronic heart failure patients, but the relation with NO has not been studied. Therefore, we investigated the roles of ANP, BNP, and NO in relation to cGMP in the blood during worsening and improvement of chronic heart failure. The subjects were 25 patients who were hospitalized in our hospital for acute worsening of chronic heart failure. Plasma concentrations of NO, norepinephrine (NE), ANP, BNP, and cGMP were measured on acute worsening (admission) and improvement (discharge) of heart failure. The cGMP concentration on worsening showed a positive correlation with the NO concentration ($r = 0.57, P < 0.01$), but no correlations with ANP or BNP were observed. The cGMP concentration on improvement showed no correlation with the NO concentration, but a positive correlation with ANP ($r = 0.69, P < 0.001$) and BNP ($r = 0.67, P < 0.001$). No correlation was observed between the NO and NE concentrations. We also studied serious cases of NYHA IV and mild cases of NYHA II to III. The cGMP concentration in the serious group showed a positive correlation with the NO concentration but no correlations with ANP or BNP concentrations on worsening. However, in the mild group, the cGMP concentration during worsening showed positive correlations with both the NO and BNP concentrations. On improvement, the cGMP concentration showed no correlation with the NO concentration but positive correlations with both the ANP and BNP concentrations in both the severe and mild groups. The results suggest the possibility that cGMP is produced mainly by NO during worsening, and by ANP and BNP rather than NO during improvement of chronic heart failure. (Jpn Heart J 2003; 44: 713-724)

Key words: Cyclic GMP, Nitric oxide, Atrial natriuretic peptide, Brain natriuretic peptide, Chronic heart failure

Nitriuretic peptide is useful in evaluation of the prognosis of chronic heart failure.1-5) Tsutamoto, et al considered the peripheral blood concentration of...
cGMP as an index of biological activity and investigated the prognosis of chronic heart failure patients based on the relation between the cGMP concentration and ANP and BNP concentrations. In the chronic heart failure survival and deceased groups, a positive correlation was observed between the cGMP and ANP concentrations, but the linear gradient showed a significant decrease in the deceased group to one third of that in the survival group. However, the cGMP and BNP concentrations did not show any definite correlation in the deceased group but a significant positive correlation was present in the survival group. cGMP is an intracellular second messenger not only for ANP and BNP but also for NO, although the relation between NO and cGMP has not been studied. Therefore, we investigated the involvement of ANP, BNP, and NO concentrations with respect to the blood levels of cGMP during worsening and improvement of chronic heart failure.

**METHODS**

**Subjects:** The subjects were 25 patients hospitalized for acute worsening of chronic heart failure. They consisted of 11 men and 14 women with a mean age of 74. Informed consent was obtained from all patients for participation in this study. Five had dilated cardiomyopathy (DCM), nine ischemic heart disease, eight valvular heart disease, and three other diseases. Renal failure patients with serum creatinine values of 3.0 unit or higher and patients with acute myocardial infarction within 3 months of onset were excluded.

Severe and mild cases were classified by NYHA criteria. NYHA stage IV patients were placed in the severe group and NYHA II to III patients in the mild group. The severe group consisted of 16 patients and the mild group 9 patients. NO, ANP, BNP, NE, and cGMP concentrations were measured on admission (worsening stage) and in the early morning after fasting at discharge (improvement stage).

**Sample measurement methods:** Samples for measurement of ANP and BNP concentrations were immediately placed in blood sampling tubes containing aprotinin (500 kallikrein activator units/mL) after drawing blood. The tubes were placed in ice and centrifuged at 4°C. The ANP concentration was measured with a specific immunoradiometric assay for human ANP using a commercial kit (Shionoria ANP, Shionogi and Co., Ltd.) and the BNP concentration was measured with a specific immunoradiometric assay for human BNP using a commercial kit (Shionoria BNP, Shionogi). The samples for measurement of the cGMP concentration were immediately placed in blood sampling tubes containing EDTA (5 mmol/L) after drawing blood. The tubes were placed in ice and centrifuged at 4°C. The plasma cGMP concentration was measured by radioimmunoas-
say with a commercial kit (Yamasa Shoyu Co., Ltd). The samples for measurement of NE were immediately placed in blood sampling tubes containing EDTA (5 mmol/L) after drawing blood. The tubes were centrifuged at 4°C for 15 minutes at 3,000 rpm. The serum was kept frozen at -30°C until measurement by HPLC. The samples for measurement of NO were centrifuged immediately after drawing blood. Protein was removed using the cold ethanol precipitation method. Nitrate (NO3-) was reduced by vanadium dichloride using the purge vessel method and NO was measured by chemiluminescence using a Sievers NO Analyzer. (Sievers Instruments Inc. Boulder Colorado, USA)

**Statistical analysis:** All values are expressed as the mean ± SEM. Pearson's coefficient of correlation was used in the analysis of correlation among the factors. Significant differences were tested using ANOVA when comparing worsening and improvement, and \( P < 0.05 \) was taken as significant.

**RESULTS**

The NO concentration decreased significantly from 69.8 ± 51.7 µmol/L on worsening to 24.4 ± 11.8 µmol/L on improvement (\( P < 0.01 \)) (Figure 1A). The ANP concentration also decreased significantly from 195.4 ± 178.1 pg/mL on worsening to 76.7 ± 68.8 pg/mL on improvement (\( P < 0.001 \)), (Figure 1B) as did the BNP concentration from 1086.9 ± 1026.9 pg/mL on worsening to 289.6 ± 361.8 pg/mL (\( P < 0.01 \)) (Figure 1C). The cGMP concentration was 7.34 ± 5.46 pg/mL on worsening and 6.33 ± 3.87 pg/mL on improvement, showing no significant difference (Figure 1D). The NE concentration decreased significantly from 1483.6 ± 940.7 pg/mL on worsening to 519.8 ± 360.6 pg/mL on improvement (\( P < 0.001 \)) (Figure 1E). The cGMP and NO concentrations on worsening showed a positive correlation at \( r = 0.57 \) (\( P < 0.01 \)) (Figure 2A). No correlations were observed between the cGMP concentration and the ANP or BNP concentrations (Figure 2B, C). The NO concentration also showed no correlation with the ANP or BNP concentration although there was a positive correlation between the ANP and BNP concentrations on worsening (\( r = 0.76, P < 0.0001 \)) (Figure 2 D,E,F).

The correlations of the cGMP concentration with the NO, ANP and BNP concentrations on improvement were examined (Figure 3). The cGMP and NO concentrations showed no correlation on improvement (Figure 3A). The cGMP concentration showed a positive correlation with the ANP concentration (\( r = 0.69, P < 0.001 \)) (Figure 3B) and with the BNP concentration (\( r = 0.67, P < 0.001 \)) (Figure 3C). The NO concentration did not show any correlation with the ANP concentration (Figure 3D) or the BNP concentration (Figure 3E) on improve-
Figure 1 Comparison of parameters (NO, ANP, BNP, cGMP and NE) on worsening and improvement of heart failure. Values are mean ± SEM. *** = P < 0.001, ** = P < 0.01, * = P < 0.05. A significant decrease was observed on worsening of heart failure in all except cGMP.

Figure 2 (A)(B)(C) Correlation of cGMP with NO, ANP and BNP on worsening of heart failure. A positive correlation existed only between cGMP and NO. (D)(E)(F) Correlation of NO with BNP and ANP on worsening of heart failure. There was no correlation between NO and BNP or ANP.
Figure 3  (A)(B)(C) Correlation of cGMP with NO, ANP and BNP on improvement of heart failure. A positive correlation existed between cGMP and BNP or ANP.  

(D)(E)(F) Correlation of NO with BNP and ANP on improvement of heart failure. There was no correlation between NO and BNP or ANP.

Figure 4  Comparison of parameters (NO, ANP, BNP, cGMP and NE) in mild and severe groups on admission. Values are mean ± SEM. * = P < 0.05.
ment, but the ANP and BNP concentrations showed a positive correlation \(r = 0.91, P < 0.0001\) (Figure 3F) on improvement, the same as on worsening. When the patients were assigned to severe and mild groups, the ANP, BNP, and NE concentrations were significantly higher in the severe group than the mild group when compared with the worsening stage \(P < 0.05\) (Figure 4), but the NO and cGMP concentrations showed no significant differences. No significant differences were observed in any of the parameters between the severe and mild groups on improvement. On worsening, the cGMP and NO concentrations showed a positive correlation \(r = 0.53, P < 0.05\), but the cGMP concentrations and ANP or BNP concentrations showed no correlation in the severe group (Figure 5A). However, in the mild group, the cGMP and NO concentrations showed a positive correlation \(r = 0.70, P < 0.05\) (Figure 5B) and the cGMP and BNP concentrations also showed a positive correlation \(r = 0.78, P < 0.05\) (Figure 5C) but the cGMP and ANP concentrations showed no correlation. On improvement the cGMP concentration showed no correlation with the NO concentration (Figure 6A, D), but positive correlations with ANP and BNP concentrations were found in both the severe and mild groups [severe group: ANP \(r = 0.72, P < 0.001\) (Figure 6B), BNP \(r = 0.68, P < 0.01\) (Figure 6C); mild group: ANP \(r = 0.68, P < 0.05\) (Figure 6E), BNP \(r = 0.74, P < 0.05\) (Figure 6F)].

**Figure 5** Correlation of cGMP with NO, ANP and BNP in mild and severe groups on worsening. A positive correlation was found between cGMP and NO not only in the severe group but also in the mild group. There was a positive correlation between cGMP and BNP.
Many neurohumoral factors are increased in patients with heart failure and are related to the severity of heart failure.\(^{10-12}\) In the present study, the concentrations of ANP, BNP, NE, and NO decreased from worsening to improvement. Both the natriuretic peptide family and NO mediate their physiological actions through the second messenger cGMP. Therefore, the cGMP concentration should show a marked increase on worsening and a decrease on improvement, but the cGMP concentration actually showed no change between worsening and improvement. Although on worsening the ANP and BNP concentrations were both high, no correlation with cGMP was observed although the cGMP and NO concentrations were correlated. When the total cGMP concentration derived from NO, ANP, and BNP was considered, the relation became \(c_{\text{GMP}}^{\text{total}} = c_{\text{GMP}}^{\text{NO}} + c_{\text{GMP}}^{\text{ANP}} + c_{\text{GMP}}^{\text{BNP}}\) (\(c_{\text{GMP}}^{\text{total}}\): total of cGMP, \(c_{\text{GMP}}^{\text{NO}}\): cGMP produced by NO, \(c_{\text{GMP}}^{\text{ANP}}\): cGMP produced by ANP and \(c_{\text{GMP}}^{\text{BNP}}\): cGMP produced by BNP). Since only cGMP and NO showed a correlation on worsening, the cGMP produced by ANP and BNP could be disregarded and it was therefore considered that cGMP on worsening was mainly derived from NO. Therefore, production of ANP and BNP increases on worsening of heart failure, but it appears that the signal trans-

**DISCUSSION**

![Figure 6](image-url)  

*Figure 6* Correlation of cGMP with NO, ANP and BNP in mild and severe groups on improvement. A positive correlation was found between cGMP and ANP or BNP not only in the severe group but also in the mild group. However, there was no correlation between cGMP and NO.
mission is interrupted at the receptor level or the site of guanylate cyclase, ie, down-regulation of the receptor occurs\textsuperscript{13,14} or the function of guanylate cyclase is suppressed.

On improvement of heart failure, correlations appeared between the cGMP concentration and the ANP and BNP concentration, but not with the NO concentration and the NO concentration was reduced more on improvement than on worsening. Therefore, cGMP on improvement was assumed to be derived mainly from ANP and BNP. This indicated that the compensating vasodilatation effects by NO required during worsening were no longer needed during improvement and up-regulation of the natriuretic peptide receptors occurred or the functional suppression of guanylate cyclase was improved.

In the study on the severe and mild groups, the ANP, BNP, and NE concentrations were all significantly higher in the severe group than in the mild group on worsening as reported previously. In an examination of the correlation between the cGMP concentration and the NO, ANP and BNP concentrations (Table), the same results observed in all patients (in combined severe and mild groups) were obtained on both worsening and improvement in the severe group, but in the mild group, the relation was different in part from that obtained from all patients. A correlation between cGMP and NO concentrations but not between cGMP and ANP concentrations was observed on worsening in the mild group. However, the cGMP and BNP concentrations were correlated on worsening in the mild group. These findings indicated that receptor down-regulation of BNP did not occur or the function of guanylate cyclase was not suppressed in mild cases.

\textbf{Table}  \textit{Correlation of cGMP with NO, ANP and BNP in Mild and Severe Groups}

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<th>All patients</th>
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<tr>
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<tr>
<td>cGMP ANP</td>
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<td>cGMP BNP</td>
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<th>On worsening</th>
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<tr>
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<td>ANP</td>
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<td>ANP</td>
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ANP and BNP both produce cGMP via specific receptors and reduce intracellular Ca\textsuperscript{2+}. As a result, they show physiological actions such as vasodilatation and inhibition of cell growth,\textsuperscript{15} but their secretory mechanisms are different. The differences in the relations of the kinetics of cGMP, ANP, BNP, and NO on worsening and improvement might involve differences in the secretory mechanisms of these factors.

The natriuretic peptide family consists of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). ANP is secreted mainly by the atria of the heart and BNP mainly by the ventricles, but as heart failure progresses, the secretion of both peptides from the ventricles shows marked increases.\textsuperscript{16-18} ANP is synthesized mainly in the cardiac atria and stored in the form of granules. It causes elongation of cardiac atrial muscle by increasing the atrial pressure and the ANP in the granules is immediately released into the bloodstream. The secretion is due to the so-called regulated pathway. When the atrial load conditions are maintained thereafter, protein synthesis and secretion occurs in the ventricles. However, BNP differs from ANP in that very little is stored as granules and the BNP that is synthesized is released continuously into the blood (constitutive pathway).

When a ventricular load occurs, the amount of BNP mRNA expressed is further increased by elongation of the ventricular muscle, protein synthesis increases and the concentration in the blood also rises. Mobilization by ANP is delayed, but overall, the increase is several thousand times the normal value in some cases.\textsuperscript{18} The difference in this secretion pattern of ANP and BNP appears to be related to the different involvement in cGMP production on worsening and on improvement and results in a difference between the serious and mild groups.

The above results suggest that BNP is more useful as a highly specific marker of heart failure conditions than ANP. This is because the same results for ANP were not obtained in the study of severe and mild groups as in the study on all patients, but in the study of the mild group, the BNP values were the same as those on improvement and in the severe group they were the same as those on worsening. Therefore, BNP can serve as a marker that can differentiate small differences. In previous studies, NE and left ventricular ejection fraction were examined as useful markers for evaluation of the prognosis and conditions of heart failure, but at present, the most useful substance is considered to be BNP.

The signal transmission systems for ANP and BNP, which are disturbed during worsening of heart failure, show normal transmission during improvement. In a study by Tamura, et al\textsuperscript{19} using knockout mice, it was shown that BNP had not only acute effects but also functioned as an antifibrotic agent involved in myocardial remodeling. Because the prognosis is poor in patients with high BNP values, it appears that myocardial fibrosis is exacerbated by high BNP and the production
of BNP is increased by the compensating inhibition of myocardial fibrosis. The significance of recovery of BNP signal transmission on improvement appears to be an antifibrotic action in the myocardium. However, this action is not considered to be sufficient in severe patients with high BNP values. In the future, the possibility that administration of BNP and administration of a neural endopeptidase inhibitor in the chronic stage are connected to the prevention of myocardial remodeling and improvement of prognosis will have to be studied.

In worsening heart failure, the cGMP and NO concentrations showed a positive correlation (Table), but on improvement, the cGMP and NO concentrations were not correlated, although the cGMP concentration and ANP and BNP concentrations showed a positive correlation (Table). Because of the relation between NO and cGMP concentrations on worsening, NO is considered to act as the main control mechanism via cGMP during worsening of heart failure. NO is produced by L-arginine and NO synthase (NOS). NOS has three isoforms: nNOS in the nervous system, eNOS expressed in the vascular endothelial cells, and iNOS expressed mainly in inflammatory cells such as leukocytes. NO increases production of cGMP by activation of soluble guanylate cyclase, decreases intracellular Ca\(^{2+}\), and shows physiological actions such as vasodilatation,\(^{20}\) a negative inotropic action,\(^{21}\) and an inhibitory action on cell growth.\(^{15}\) It has been reported that NO concentrations increase as heart failure becomes more severe.\(^{22}\) From these results, it appears that NO is generally considered to be involved in worsening of the pathophysiology of heart failure. However, many drugs with a positive inotropic action increase the mortality of heart failure patients, while drugs with negative inotropic action such as beta-blockers are useful in improving the prognosis.

NO has also been reported to improve the dilatation capacity of the left ventricle.\(^{23}\) Therefore, the most recent consideration is that reduction of cardiac contractility and improvement of dilatation capacity by NO causes a decrease in myocardial oxygen consumption and that NO has a direct coronary artery dilatation action, which protects the myocardium. In our results, ANP and BNP signal transmission was interrupted on worsening of heart failure but the production of cGMP by NO was maintained. It appears that NO is produced by the vascular endothelium due to hemodynamic changes on worsening of heart failure and this acts as a compensatory mechanism in heart failure. However we experienced one DCM patient who died of uncontrolled heart failure and whose concentration of NO continuously increased. It is believed that NO has toxic effects on the myocardium when the concentration of NO continues to rise and exceeds a certain level. Thus, a continuous increase in NO was suggested to have a deteriorating effect on the prognosis and might be used to predict the prognosis.
Conclusion: The findings suggest the possibility that cGMP is produced mainly by NO on worsening of heart failure and by ANP and BNP rather than by NO on improvement of heart failure.

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REFERENCES